Original Research Article

Acute, Sub-acute and Chronic Toxicity Evaluation of Aqueous Extract of the Aerial Parts of *Leonurus cardiaca* in Normal Female Wistar Albino Rats Per OECD 425 TG

Abstract

This study graded the acute, sub-acute, and chronic toxicity of aqueous extract of the aerial parts of *Leonurus cardiaca* in non-pregnant female wistar albino rats. Fifty wistar albino rats weighing between 180 and 200g were used for this study. The rats were grouped into 10 groups of five rats per group. Group 1 served as control while group 2-11 were orally administered aqueous extract of *Leonurus cardiaca* at 166, 250, and 500mg/kg for 7, 14, and 21 days. All haematological and biochemical parameter were determined using standard methods. The plasma RBC, Hb, MCV, and WBC of the control were 5.33±0.01 x 10^{12}/L, 13.54±0.01 g/dl, 42.16±0.01 fl, and 1.83±0.01 X 10^{9}/L respectively. The plasma RBC, Hb, MCV, and WBC of the rats treated with 500mg/kg of the extract were 10.93±0.01x 10^{12}/L, 19.24±0.01g/dl, 28.33±0.01fl, and 1.41±0.01 x 10^{9}/L respectively for 21 days and were significantly different from the control at $p \leq 0.05$. The plasma Na^{+}, K^{+} , Cl^{-}, HCO_3 , creatinine, and urea levels treated with extract at 500mg/kg for 21 days were 167.97±0.01 01mmol/l, 7.85±0.0101mmol/l, 164.24±0.0101mmol/l, 40.84±0.0101mmol/l, 1.16±0.0101mmol/l, and 5.68±0.02 01mmol/l respectively and were significantly different from the control. The plasma ALT, ALP, and AST activities treated with extract at 500mg/kg for 21 days were 146.24±0.02U/L, 77.33±0.01U/L, and 168.71±0.01U/L respectively which were significantly different from the control. Enhancement on heart, kidney and liver tissues occurred after treatment with 500mg/kg of the extract for 21 days. The significantly improved effects observed on all assayed parameters were expressive that aqueous extract of the aerial parts of *Leonurus cardiaca* extract is safe at 500 mg/Kg.

Keywords: HPLC, G.C, heart, kidney, liver, *Leonurus cardiaca*, plasma

1.0 INTRODUCTION

Medicinal plants elicit several therapeutic virtues, but they are not free from any danger of intoxication. Researchers have pointed out the potential toxicity, as well as the risks associated with the use of certain species of plants and vegetables [1]. Some medicinal plants that toxic cause damage to vital organs such the liver, kidney, heart, and the brain, which has made studies

on the safety and effectiveness of medicinal plants of great concern has in order to guarantee their usage [2].

Toxicity can be considered as complete indication of poisonous impact which present the state of adverse effects facilitated by the interaction of toxicants and cellular components of an organism. Toxicity can occur within or around cell membranes or on the periphery of tissues including at the extracellular matrix [3].

Leonurus cardiaca (Motherwort) is probably best known as a uterine stimulant, which is where its name comes from. It is used for painful or delayed periods, and in the last few weeks of pregnancy to prepare for childbirth. It is also known to ease symptoms of menopause [4]. It is used by trado-medical practioners for treatment of heart failure in traditional medicine. *Leonurus cardiaca* is a Latin name which means something like "lion's heart," and refers to motherwort's use as a cardiovascular tonic in traditional system of medicine. It strengthens the heart and can treat heart palpitations and irregularities, especially where those are associated with anxiety and tension. Preparation from *Leonurus cardiaca* is used as teas (Infusion). Since the parts of motherwort that are used medicinally are leaves, flowers, and stems, it requires the infusion method [5]. However, there are no existing literature regarding the toxicological implication of *Leonurus cardiaca*, hence the need to experiment on acute, sub-acute and chronic toxicity studies on the aerial parts of the plants becomes imperative, which is the focus of this study.

2.0 MATERIALS AND METHODS

2.1 Chemical/Reagents

All chemical/reagents used for this study were purchased from commercial industries and the manufacturers' standard methods and procedure were strictly followed with regard to this study

2.2 Source and Identification of Plant Material

The fresh aerial parts of *Leonurus cardiaca* (LNC) were harvested from Idema Community, in Ogbia Local Government Area of Bayelsa State, Nigeria. The plant sample was identified and authenticated by Dr. Ekeke Chimezie at the Herbarium Unit of the Department of Plant Science and Biotechnology (PSB), University of Port Harcourt. The sample was registered with Voucher Number UPH/P/203.

2.3 Aqueous Extraction of the Aerial Parts of *Leonurus cardiaca*

The fresh aerial parts of *Leonurus cardiaca* were harvested from Idema Community Ogbia Local Government Area, Bayelsa State, Nigeria. The aerial parts were washed with clean running tap water and air-dried under shade for five weeks. The dried aerial parts of the plant were pulverized into coarse powder. One hundred and five grams (105 g) of the powdered sample was macerated in 500 ml of distilled water at room temperature for 72 hours. The mixture was filtered using a Whatzman filter paper grade 1 (542 mm) and the filtrate condensed and evaporated to dryness using a rotary evaporator (RM2235 Leica Biosystems USA) and water bath at 50˚C. The extract which weighed 79 g was stored in air-tight containers in a refrigerator until when required for treatments.

2.4 Preliminary Phytochemicals Screening

The aqueous extract of the aerial parts of *Leonurus cardiaca* were screened for the presence of various phytoconstituents according to the procedure described by Khandelwal [6].

2.5 Source of Experimental Wistar Albino Rats

Fifty (50) adult non-pregant albino Wistar rats (Rattus norvegicus) weighing 120 and 125 g were purchased from the Biochemistry Animal House, University of Nigeria Nsukka (UNN) and be acclimatized for two weeks, giving free access to rat feed and distilled water (DW). The rats were kept in clean plastic cages in well ventilated room, fed with standard animal feeds, produced by Grand Cereals and Oil Mills Ltd., Port Harcourt, and water *ad libitum*. The animals were handled with care, according to the principles and standard protocols for the use of laboratory animals for experiments.

2.6 Determination of Acute Oral Toxicity and Median Lethal Dose (LD_{50}) of Aqueous Extract of the Aerial Parts of *Leonurus cardiaca*

The LD_{50} values of aqueous extract of the aerial parts of *Leonurus cardiaca* was determined using the up and down method of Organization of Economic Cooperation and Development (UPD/OECD 425) (1998), which received regulatory approval by the regulatory body (PISC, 2017). Following this method, twenty (20) female, non-pregnant wistar albino rats were obtained and grouped into four groups, five rats per group. The wistar albino rats in each group were sequentially dosed one at time, using four graded doses (1250, 2500, 3750 and 5000 mg/kg b.wt) of aqueous extract of the aerial parts of *Leonurus cardiaca*. Rats in group one were first administered the extract at 1250 mg/kg b.wt once and observed for two days for signs of toxicity and mortality. After two days of observation, toxicity and mortality did not occur, hence the initial dose was doubled (2500 mg/kg b.wt) and administered to rats in group two and same continued until 5000mg/kg. B.wt, based on the up and down method approved by Peta International Science Consortium Ltd in 2017 reported by Enegide *et al.* [7] and Earnest *et al*. [8]. The median lethal dose was calculated using the formula as explained by Enegide *et al.* [7] shown below:

$LD_{50} = \frac{[M_0 - M_1]}{2}$

Where M_0 = Highest dose of test substance that gave no mortality,

M_1 = Lowest dose of test substance that gave mortality.

2.7 Animals and Approval from Animal Ethical Committee

Healthy nulliparous and non-pregnant female wistar albino rats (120–125 g) between 8–10 weeks were used for all the experiments in the present study. The animals were maintained under standard husbandry conditions in the animal house of 'College of Pharmaceutical Sciences, University of Port Harcourt, Nigeria (temperature 25 ± 2 °C) in a natural light-dark cycle and fed with standard rodent diet and water ad libitum. Ethical committee clearance was obtained from IAEC (Institutional Animal Ethics Committee) of UPH/CEREMAD/REC/MM72/035 .Ref. No. G2018/PHD/BCH/FT/009).

2.5 Experimental Design

Fifty (50) wistar albino rats weighing between 180 and 200g were used for this study. They were be purchased from the Biochemistry Animal House, University of Nigeria Nsukka (UNN) and be acclimatized for two weeks, giving free access to rat feed and distilled water (DW). The rats were then be grouped on basis of body weight into ten groups five rats per group and treated as follows:

Group 1: Normal control: Feed + H_2O.

Group 2: 166 mg/kg/b.wt Extract+ feed+ H_2O for 7days

Group 3: 166 mg/kg/b.wt Extract+ feed+ H_2O for 714 days

Group 4: 166 mg/kg/b.wt Extract+ feed+ H_2O for 21 days

Group 5: 250 mg/kg/b.wt Extract+ feed+ H_2O for 7days

Group 6: 250 mg/kg/b.wt Extract+ feed+ H_2O for 14 days

Group 7: 250 mg/kg/b.wt Extract+ feed+ H_2O for 21 days

Group 8: 500 mg/kg/b.wt Extract + feed+ H_2O for 7 days

Group 9: 500 mg/kg/b.wt Extract + feed+ H_2O for 14 days

Group 10: 500 mg/kg/b.wt Extract + feed+ H_2O for 21 days

Exactly, 24 hours after the last day of oral treatment with the extract, the rats were humanly sacrificed through cervical dislocation, blood sample were be collected for biochemical assays and estimation of haematological profile. The heart and kidney were harvested and were cut into two equal halves for each organ. Each half of the organs were homogenized for estimation of oxidative biomarkers for heart and kidney, while the other halves were subjected to histological examination.

2.6 Biochemical Analysis

Different biochemical parameters were measured using ELISA and Randox kits in a biochemical analyzer. The parameters observed for liver function parameters observed are aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphate, bilirubin, albumin and, and total protein. Plasma cardiac markers were cardiac troponin T (CTn-T), cardiac troponin I (CTn-I), interleukin 6 (IL-6) and C-reactive protein (CRP). The plasma enzymes biomarkers of cardiac were creatine kinase (CK), alanine amino transferase (ALT), alkaline phosphatase (ALP), aspartate transaminase (AST) and lactate dehydrogenase (LDH) while the Kidney homogenate biomarkers were sodium ion (Na^+), potassium ion (K^+), chloride ion (Cl^-),

bicarbonate ion (HCO_3^-), creatinine and blood urea nitrogen (BUN). The oxidative plasma cardiac and kidney homogenate biomarkers determined were malondialdehyde (MDA), glutathione (GSH), glutathione peroxidase (PDx), superoxide dismutase (SOD) and catalase (CAT). Lipid Profile determined were total cholesterol, triglyceride, high density lipoprotein (HDL), very low density lipoprotein (VLDL), and low density lipoprotein (LDL) were observed.

2.7 Haematological Analysis

Blood samples from non-pregnant female wistar albino rats (both treated and vehicle control groups) were collected into EDTA containing tubes for haematological study. Hemoglobin (Hb), total RBC, packed cell volume (PVC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), hematocrit, white blood cells (WBC) count were determined.

2.8 Histopathological Analysis of Heart, Kidney, and Liver Tissues

The heart, kidney, and liver isolated from sacrificed non-pregnant wistar albino rats were fixed in 10% formalin, then after processing embedded in paraffin wax. Paraffin sections were made at 5 mm and stained with hematoxylin and eosin. The slides were studied under a light microscope and captured the magnified images of tissues structure for further study.

2.9 Statistical Analysis

All Data are represented as means ± standard deviation (M±S) were analyzed using Statistical Package foe Social Sciences (SPSS) for window version 17.0 USA. Descriptive statistics was done by one way analysis of variance (ANOVA) and multiple comparison was done using Turkey Post hoc at ($p \leq 0.05$) confidence interval.

3.0 RESULTS AND DISCUSSION

Medicinal plants are been used many years ago to treat various diseases. One such medicinal plant is *Leonurus cardiaca,* which has been used in Latin and Nigeria system of traditional medicine as curative agent. These may lead to toxicity of the plant constituents, as it is the dose that makes the drug a poison. Hence, this study was designed to grade and evaluate the toxic effect of the plant by oral route through modification of 425 toxicity guidelines in order to achieve the purpose the study.

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The medicinal properties and toxicity effects engendered by medicinal plants such as *Leonurus cardiaca* are attributed to the presence of bioactive ingredients in the roots and aerial parts of such plants [10]. In this study, phytochemical screening and quantification of the aerial parts of *Leonurus cardiaca* showed the presence of terpenoids, phenolic acids, anthraquinones, anthocyanins, saponins, tannins, phytates, flavonoids, cynogenic glycosides, alkaloids, glycosides, and essential oils as shown in Table 1, which could be responsible for the medicinal properties of the reported by [11, 12, 13, 14].

Table 1 The preliminary phytochemical screening and quantification of the extracts of the aerial parts of *Leonurus cardiaca* revealed the presence of different chemicals which are been listed in Table 1.

Phytochemical	Concentration (mg/100 g)
Terpenoids	26.19×10^{-1}
Phenolic acids	506.33
Saponins	62.33
Cyanogenic glycosides	118.03
Glycosides	16.17
Anthocyanins	56.53
Alkaloids	1.31
Flavonoids	7.31
Sterols	5.91
Tannins	426.49
Phytate	69.12
Essential oils	100%

The toxic outcomes and mortality can be determined by the clinical signs and symptoms among other toxicity markers [15]. In this present study, aqueous extract of the aerial parts of *Leonurus cardiaca* was did not caused mortality at 5000 mg/kg b.wt based on the OECD 225 TG guidelines. Also, the wistar albino rats exposed to the extracted at four graded doses particularly at 166, 250, and 500 mg/kg b.wt, did not cause mortality in all the rats tested as shown in Table 2 and 3. Behavioral studies like respiration, increased somatomotor activity and itching were been observed during the first 30 min after the aqueous extract of the aerial parts of *Leonurus cardiaca* was orally administered. It suggested that the extract did interfere with the normal physiological and biochemical processing of all the nutrients like carbohydrate, proteins and fats are been metabolized appropriately within the rats since nutrients that play a major role in physiological function [15, 16, 17].

Table 2 Effect of different doses of aqueous extract of the aerial parts of Leonurus cardiaca on toxicity parameters in normal non-pregnant female wistar albino rats for 7 days of exposure.

Parameter	166 mg/kg b.wt	250 mg/kg b.wt	500 mg/kg b.wt
Coma	A	A	A
Convulsion and tremor	A	A	A
Eye and bleeding	N	N	N
Faeces consistency	N	N	N
Itching	N	N	P
Mucous membrane	N	N	N
Mortality	A	A	A
Respiration	N	N	N
Salivation	N	P	P

A=absent, N=Normal, P= present

Table 3 Table Effect of different doses of aqueous extract of the aerial parts of *Leonurus cardiaca* on toxicity parameters in normal non-pregnant female wistar albino rats for 21 days of exposure

Parameter	166 mg/kg b.wt	250 mg/kg b.wt	500 mg/kg b.wt
Coma	A	A	A
Convulsion and tremor	A	P	P
Sleep	N	N	N
Faeces consistency	N	N	N
Itching	N	P	P
Mucous membrane	N	N	N
Mortality	A	A	A
Respiration	N	P	N

A=absent, N=Normal, P= present

Hematological parameters are perceptive indicators of the physiological changes in retort to any toxic stress or environmental pollutant in animals (Jain *et al.*, 2009). Blood platelet plays a vital role in Haemostasis (Li *et al.*, 2008). In this investigation, remarkable increases occurred on the plasma RBC, Hb, MCV, MCH, haematocrit, and WBC, after exposure to the extract at 166, 250, and 500 mg/kg b.wt for 7, 14 and 21 days as shown in Table 4. The remarkable increases on the

haematological profile of the extract treated rats sugguesr that there was some influence of the extract on the haematopoiesis pathway.

Table 4 Effect of *aqueous* of the aerial part of *Leonurus cardiaca* on the plasma haematological parameters of normal wistar albino rats

Treatment	RBC (X 10^{12}/L)	Haemoglobin (g/dl)	Haematocrit (l/L)	MCV (fl)	MCH (pg)	WBC (X 10^{9}/L)
Normal control	5.33±0.01	13.54±0.01	0.55±0.01	42.16±0.01	50.24±0.01	1.83±0.01
166mg/kg for 7 days	5.65±0.01[a]	13.84±0.02[a]	0.86±0.01[a]	42.01±0.01[a]	50.44±0.01[a]	1.80±0.00[a]
166mg/kg for 14 days	5.95±0.01[a]	14.85±0.01[a]	0.93±0.02[a]	41.94±0.01[a]	50.94±0.01[a]	1.77±0.01[a]
166mg/kg for 21 days	6.25±0.02[a]	15.05±0.01[a]	1.04±0.01[a]	41.72±0.01[a]	51.44±0.01[a]	1.72±0.01[a]
250mg/kg for 7 days	7.33±0.01[a]	16.45±0.01[a]	1.64±0.01[a]	38.83±0.01[a]	62.43±0.01[a]	1.65±0.01[a]
250mg/kg for 14 days	7.85±0.02[a]	16.94±0.01[a]	1.85±0.02[a]	38.44±0.01[a]	62.93±0.01[a]	1.61±0.01[a]
250mg/kg for 21 days	8.34±0.01[a]	17.85±0.01[a]	2.15±0.02[a]	38.13±0.01[a]	63.44±0.01[a]	1.57±0.01[a]
500mg/kg for 7 days	10.24±0.01[a]	18.44±0.02[a]	2.65±0.01[a]	28.84±0.01[a]	69.24±0.01[a]	1.51±0.01[a]
500mg/kg for 14 days	10.65±0.01[a]	18.95±0.02[a]	2.85±0.01[a]	28.53±0.01[a]	69.82±0.01[a]	1.46±0.01[a]
500mg/kg for 21 days	10.93±0.01[a]	19.24±0.01[a]	3.06±0.02[a]	28.33±0.01[a]	71.04±0.01[a]	1.41±0.01[a]

Values are reported as mean ± standard error of mean (M±SEM) (n =5). Values with similar superscript letters indicate significant differences ($p \leq 0.05$) when compared with the control group.

Treatment with aqueous extract of the aerial parts of *Leonurus cardiaca* at 166mg/kg b.wt for 14 and 21 days, resulted in significant decreases on the CTn-T, CTn-I, IL-6 and c-reactive protein in group 4-5 when compared to the positive and negative control (Table 5.).

Significant decreases ($p<0.05$) on the CTn-T, CTn-I, IL-6 and c-reactive protein occurred in rats after treatment with *Leonurus cardiaca* extract at 250mg/kg b.wt. Treatment with the extract 250mg/kg b.wt produced more significant decreases ($p<0.05$) on the CTn-T, CTn-I, IL-6 and c-reactive protein when compared with the control (Table 5.). However, treatment at 500mg/kg b.wt significantly resulted in decreases ($p<0.05$) on the plasma CTn-T, CTn-I, IL-6 and c-reactive protein when compared to the control. The significant decreases observed after treatment with aqueous extract of *Leonurus cardiaca* were reflective of the ability of *Leonurus cardiaca* to enhance cardiac functioning through improvement on the neuro-cardiac protein and hormones. The significant improved effects elicited by *Leonurus cardiaca* extract agrees with the report of Kaksha *et al.* [18] on evaluation of cardioprotective effect of aqueous extract of Garcinia indica Linn. fruit rinds on isoprenaline-induced myocardial injury in Wistar albino rats.

Table 5 Effects of Aqueous extract of the aerial Parts *Leonurus cardiaca* on plasma cardiac markers in Normal Wistar Albino Rats

Treatment	CTn-T (pg/mL)	CTn- I (pg/mL)	IL-6 Level (pg/dl)	CRP (mg/ml)

Normal control	291.14±0.01	172.24±0.01	27.34±0.01	14.28±0.02
166mg/kg b.t for 7 days	279.87±0.01[a]	167.96±0.01[a]	27.24±0.01[a]	14.19±0.01[a]
166mg/kg b.t for 14 days	279.02±0.01[a]	164.92±0.02[a]	27.15±0.01[a]	14.06±0.01[a]
166mg/kg b.t for 21 days	272.74±0.01[a]	157.65±0.02[a]	27.05±0.02[a]	13.86±0.02[a]
250mg/kg b.t for 7 days	251.71±0.01[a]	141.75±0.01[a]	25.87±0.01[a]	13.66±0.03[a]
250mg/kg b.t for 14 days	241.96±0.01[a]	137.67±0.01[a]	25.12±0.02[a]	13.41±0.01[a]
250mg/kg b.t for 21 days	241.22±0.01[a]	133.73±0.01[a]	22.74±0.01[a]	13.21±0.01[a]
500mg/kg b.t for 7 days	231.07±0.02[a]	128.86±0.02[a]	22.12±0.02[a]	11.72±0.03[a]
500mg/kg b.t for 14 days	210.77±0.01[a]	128.04±0.01[a]	19.84±0.01[a]	11.40±0.01[a]
500mg/kg b.t for 21 days	195.23±0.01[a]	125.45±0.01[a]	19.05±0.01[a]	11.26±0.02[a]

Values are reported as mean ± standard error of mean (M±SEM) (n =5). Values with similar superscript letters indicate significant differences ($p \leq 0.05$) control group.

Significant increases ($p<0.05$) were observed after treatment with the extract at 166mg/kg b.wt dose for 7, 14, and 21 days in comparison to the control (Table 6), which is reflective that the extract at 166mg/k elicit improved effect n kidney function in the rats. However, significant increases on kidney plasma sodium) (Na^+), potassium (K^+), chloride (Cl^-), bicarbonate (HCO_3^-), creatine and urea concentrations were observed at 250mg/kg b.wt treatment for 7, 14, and 21 days in comparison to the control values (Table 7). More so, a more significant increases ($p<0.05$) on the plasma sodium) (Na^+), potassium (K^+), chloride (Cl^-), bicarbonate (HCO_3^-), creatine and urea concentrations were observed at 500mg/kg b.wt treatment for 7, 14, and 21 days in comparison to the control values (Table 6). The significant improvement or increases observed following treatment with aqueous extract of the aerial parts of *L. cardiaca* is suggestive that *Leonurus cardiaca* extract enhanced renal function by improving on the markers of renal function parameters of wistar rats. This findings were agreement with the report of Chinedu *et al*. [19] on the effects of ethanolic extracts of leaf, seed

and fruit of *Datura metel* on amelioration of cisplatin-induced nephrotoxicity by ethanolic extract of *Bauhinia purpurea*: An in vivo study in rats on kidney function of male albino rats.

Table 6 Effect of aqueous extract of the aerial parts of *Leonurus cadiaca* plasma kidney biomarkers concentrations in normal wistar albino rats

Treatment	Na^+ (mmol/l)	K^+ (mmol/l)	Cl^- (mmol/l)	HCO_3 (mmol/I)	Creatinine (mmol/l)	Urea (mmol/l)
Normal control	153.24±0.01	3.45±0.01	81.34±0.01	26.45±0.04	0.36±0.04	2.35±0.01
166mg/kg for 7 days	155.04±0.01[a]	3.77±0.02[a]	87.45±0.01[a]	26.81±0.02[a]	0.36±0.04	2.86±0.01[a]
166mg/kg for 14 days	158.23±0.03[a]	3.97±0.00[a]	91.45±0.01[a]	27.12±0.02[a]	0.52±0.02	3.06±0.01[a]
166mg/kg for 21 days	158.72±0.01[a]	4.13±0.01[a]	101.65±0.01[a]	23.18±0.01[a]	0.60±0.00[a]	3.55±0.01[a]
250mg/kg for 7 days	161.84±0.01[a]	4.95±0.01[a]	112.07±0.01[a]	34.23±0.02[a]	0.80±0.00[a]	4.03±0.02[a]
250mg/kg for 14 days	162.39±0.02[a]	5.09±0.00[a]	112.79±0.02[a]	34.82±0.02[a]	0.80±0.00[a]	4.36±0.01[a]
250mg/kg for 21 days	162.54±0.01[a]	5.35±0.02[a]	113.05±0.01[a]	35.09±0.01[a]	0.80±0.00[a]	4.75±0.01[a]
500mg/kg for 7 days	165.93±0.02[a]	7.05±0.02[a]	143.35±0.01[a]	40.08±0.03[a]	0.72±0.18[a]	4.94±0.01[a]
500mg/kg for 14 days	167.34±0.01[a]	7.43±0.06[a]	163.94±0.01[a]	40.48±0.02[a]	1.01±0.00[a]	5.14±0.02[a]
500mg/kg for 21 days	167.97±0.01[a]	7.85±0.01[a]	164.24±0.01[a]	40.84±0.01[a]	1.16±0.01[a]	5.68±0.02[a]

Values are reported as mean ± standard error of mean (M±SEM) (n =5). Values with similar superscript letters indicate significant differences ($p \leq 0.05$) when compared to the control group.

Also, oxidative damage is aggravated by the decrease in antioxidant enzymes activities such as superoxide dismutase, catalase (CAT), and glutathione peroxidase (GPx) which acts as a free radical scavengers in conditions associated with oxidative stress (Blokhina *et al.,* 2002). The decreases observed on the heart homogenate CAT, SOD, and GPx activities were pointers to oxidative stress condition which is in line with the report of (Blokhina *et al.,* 2002). Significant increases on the heart and kidney CAT, SOD, and GPx activities occurred after 7, 14, and 21 days after treatment with *Leonurus cardiaca* extract at 166, 250 and 500mg/kg in comparison to the control values (Table 7 and 8). The significantly increased plasma CAT, SOD and GPx activities noticed in both the heart and kidneys after exposure to *Leonurus cardiaca* extract are suggestive of the antioxidant properties of the aerial parts of *Leonurus cardiaca* against oxidative

stress in rats. This findings confirm the report of Sadowska *et al.* [20] on the immunomodulatory potential of *Leonurus cardiaca* extract in relation to endothelial cells and platelets.

Table 7 Effect of aqueous of the aerial parts of *Leonurus cardiaca* on plasma oxidative stress cardiac biomarkers activities in normal wistar albino rats

Treatment	MDA (mmol/l)	SOD (mg/g)	GPx (IU/g)	Catalase (µmol.H_2O_2mg/pro/min)
Normal control	4.95±0.01	6.00±0.00	56.24±0.01	84.78±0.59
166mg/kg b.t for 7 days	4.85±0.01[a]	6.13±0.01[a]	56.55±0.02[a]	87.45±0.02[a]
166mg/kg b.t for 14 days	4.33±0.01[a]	6.73±0.01[a]	56.84±0.01[a]	89.03±0.01[a]
166mg/kg b.t for 21 days	4.15±0.02[a]	6.93±0.01[a]	87.12±0.01[a]	89.72±0.01[a]
250mg/kg b.t for 7 days	3.83±0.02[a]	7.27±0.01[a]	91.05±0.02[a]	96.75±0.02[a]
250mg/kg b.t for 14 days	3.44±0.01[a]	7.82±0.01[a]	91.54±0.01[a]	100.26±0.01[a]
250mg/kg b.t for 21 days	3.84±0.01[a]	8.02±0.01[a]	100.04±0.01[a]	100.72±0.02[a]
500mg/kg b.t for 7 days	2.76±0.01[a]	9.39±0.02[a]	100.76±0.01[a]	101.55±0.01[a]
500mg/kg b.t for 14 days	2.54±0.01[a]	9.84±0.01[a]	101.23±0.01[a]	101.89±0.02[a]
500mg/kg b.t for 21 days	2.15±0.01[a]	10.02±0.01[a]	101.74±0.01[a]	102.13±0.01[a]

Values are reported as mean ± standard error of mean (M±SEM) (n =5). Values with similar superscript letters indicate significant differences ($p \leq 0.05$) when compared to the control group.

Table 8 Effect of aqueous of the aerial parts *Leonurus cardiaca* on the oxidative stress kidney biomarkers activities in normal wistar albino rats

Treatment	MDA (mmol/l)	SOD (mg/g)	GPx (IU/g)	Catalase (µmol H_2O_2 C/mg/pro./min)
Normal control	4.62±0.01	8.05±0.02	51.14±11.20	87.57±0.01
166mg/kg b.t for 7 days	2.45±0.01[a]	8.23±0.01[a]	62.94±0.01[a]	87.64±0.01[a]
166mg/kg b.t for 14 days	2.35±0.01[a]	8.66±0.01[a]	78.53±0.01[a]	87.95±0.01[a]
166mg/kg b.t for 21 days	2.22±0.01[a]	93.06±0.01[a]	81.53±0.01[a]	88.77±0.01[a]
250mg/kg b.t for 7 days	1.01±0.01[a]	100.77±0.02[a]	94.47±0.01[a]	104.05±0.01[a]
250mg/kg b.t for 14 days	0.87±0.01[a]	100.95±0.01[a]	100.45±0.01[a]	104.83±0.01[a]
250mg/kg b.t for 21 days	0.75±0.01[a]	101.35±0.01[a]	100.96±0.01[a]	104.97±0.01[a]
500mg/kg b.t for 7 days	0.34±0.01[a]	142.08±0.01[a]	132.33±0.01[a]	116.66±0.01[a]
500mg/kg b.t for 21 days	0.26±0.01[a]	142.86±0.01[a]	132.93±0.01[a]	116.95±0.01[a]

Values are reported as mean ± standard error of mean (M±SEM) (n =5). Values with similar superscript letters indicate significant differences ($p \leq 0.05$) when compared to the control group.

Treatment with aqueous extract of *Leonurus cardiaca* at 166, 250 and 500mg/kg b.wt for 7, 14, and 21 day resulted in a significantly decreased CK, ALT, ALP, AST, and LDH activities when compared with the control (Table 9). A more improved effects occurred at 250 and 500mg/kg b.wt treatment with the extract. The decreases noticed after treatment with *Leonurus cardiaca* extract is indicative of the potential capacity of the plant in enhancing cardiac performance through enhancement of markers of cardiac protein parameters in rats. Tayyaba *et al.* [21] reported similar results in their work on evaluating the protective potency of *Acacia hydaspica* R. Parker on histological and biochemical changes induced by cisplatin in the cardiac tissue of rats.

Table 9 Effect of aqueous extract of the aerial parts of *Leonurus cardiaca* on plasma Cardiac biomarkers activities in normal non-pregnant wistar albino rats

Treatment	C-kinase (U/L)	ALT (U/L)	ALP (U/L)	AST (U/L)	LDH (U/L)
Normal control	192.27±0.02	63.25±0.01	72.05±0.01	185.87±0.01	178.24±0.01
166mg/kg for 7 days	188.24±0.02[a]	162.73±0.01[a]	67.25±0.01[a]	156.46±0.02[a]	161.69±0.02[a]
166mg/kg for 14 days	165.24±0.02[a]	158.65±0.01[a]	61.88±0.01[a]	142.85±0.01[a]	155.33±0.01[a]
166mg/kg for 21 days	157.10±0.01[a]	145.76±0.01[a]	57.08±0.01[a]	132.05±0.01[a]	144.23±0.01[a]
250mg/kg for 7 days	150.66±0.01[a]	121.07±0.01[a]	53.87±0.01[a]	119.53±0.01[a]	132.34±0.02[a]
250mg/kg for 14 days	146.31±0.01[a]	105.64±0.01[a]	50.14±0.01[a]	100.64±0.01[a]	114.06±0.02[a]
250mg/kg for 21 days	125.43±0.01[a]	89.53±0.01[a]	47.26±0.01[a]	94.03±0.02[a]	102.06±0.02[a]
500mg/kg for 7 days	102.85±0.01[a]	73.08±0.01[a]	45.76±0.01[a]	87.02±0.01[a]	97.02±0.02[a]
500mg/kg for 14 days	97.24±0.01[a]	63.66±0.01[a]	41.25±0.01[a]	83.03±0.02[a]	92.76±0.01[a]
500mg/kg for 21 days	94.05±0.03[a]	61.76±0.01[a]	35.96±0.01[a]	74.04±0.01[a]	85.66±0.01[a]

Values are reported as mean ± standard error of mean (M±SEM) (n =5). Values with similar superscript letters indicate significant differences ($p \leq 0.05$) when compared to the control group.

Total protein, albumin, and total bilirubin are also biomarkers that can be used to predict the health status of the liver. Significant decreases on serum or plasma total protein and albumin concentration as well as increases in bilirubin levels and AST, ALP, GGT, and ALT activities are pointers to liver compromise and hepatotoxicity [22]. However, the total protein and albumin were significantly increased while bilirubin level was observed to be significantly decreased ($p<0.05$) at 166mg/kg b.wt *Leonurus cardiaca* exposure for 7, 14, and 14 days treatment in comparison to the control values (Table 10). The ALT, ALP, and AST activities were significantly decreased ($p<0.05$) at 166mg/kg b.wt of the extract for same periods of treatment. Treatment with the extract at 250 and 500mg/kg b.wt for 7, 14, and 21 days resulted in

significantly increased and decreased plasma total protein, albumin and bilirubin concentrations respectively in comparison to the control values (Table 10). The significant improvement observed following exposure to the aqueous extract particularly at doses 250 and 500 mg/kg b.wt are expressive of the capacity of the extract to enhance hepatic performance in rats. These results conformed to the report of Emmanuel *et al.* [23] on the effects of *Azadirachta indica* leaf aqueous extract on the antioxidant enzymes in paracetamol-induced hepatotoxicity in Wistar rats. Table 10 shows the effect of aqueous extract of the aerial parts of *Leonurus cardiaca* on the plasma concentrations and enzymes activities of normal wistar rats

Table 10Effect of aqueous extract of the aerial parts of *Leonurus cardiac*a on plasma liver biomarkers activities

Treatment	TP (g/L)	Albumin (mmol/L)	Total bilirubin (μmol/L)	ALT (U/L)	ALP (U/L)	AST (U/L)
Normal control	55.24±0.01	27.30±0.01	10.45±0.01	146.24±0.02	77.33±0.01	168.71±0.01
166mg/kg for 7 days	59.76±0.19[a]	29.05±0.02[a]	10.24±0.01[a]	131.84±0.02[a]	72.65±0.01[a]	162.54±0.02[a]
166mg/kg for 14 days	63.24±0.01[a]	35.74±0.01[a]	10.04±0.01[a]	124.44±0.02[a]	67.23±0.01[a]	153.70±0.02[a]
166mg/kg for 21 days	67.89±0.02[a]	39.53±0.02[a]	9.94±0.01[a]	117.24±0.01[a]	62.62±0.01[a]	149.68±0.02[a]
250mg/kg for 7 days	73.12±0.01[a]	43.03±0.02[a]	9.53±0.01[a]	97.65±0.01[a]	56.34±0.01[a]	143.24±0.02[a]
250mg/kg for 14 days	75.03±0.01[a]	48.65±0.01[a]	9.03±0.01[a]	92.03±0.02[a]	50.64±0.02[a]	137.22±0.01[a]
250mg/kg for 21 days	75.96±0.01[a]	52.14±0.01[a]	8.44±0.01[a]	85.05±0.02[a]	44.93±0.01[a]	124.22±0.01[a]
500mg/kg for 7 days	84.26±0.02[a]	64.04±0.01[a]	7.63±0.01[a]	73.35±0.02[a]	31.21±0.01[a]	115.64±0.02[a]
500mg/kg for 14 days	85.93±0.01[a]	68.31±0.07[a]	16.85±0.01[a]	157.36±0.02[a]	27.21±0.01[a]	100.85±0.01[a]
500mg/kg for 21 days	87.14±0.02[a]	72.04±0.01[a]	16.08±0.02[a]	147.33±0.01[a]	24.64±0.02[a]	96.03±0.02[a]

Values are reported as mean ± standard error of mean (M±SEM) (n =5). Values with similar superscript letters indicate significant differences ($p \leq 0.05$) when compared to the control group.

Significant increases ($p<0.05$) on the total cholesterol, HDL, VLDL, and triglyceride concentrations were observed after treatment with *Leonurus cardiac* extract at 166, 250, and 500mg/kg b.wt for 7, 14, and 21 days in comparison to the control values (Table 11). More significant increases on at 500mg/kg exposure to the *Leonurus cardiaca* extract. The dose-dependent increases on the lipid profile observed after treatment with extract were expressive of the plant is a rich source of good cholesterol and can be used in treating hypolipidaemic conditions. However, the *Leonurus cardiaca*-treated showed intact renal and hepatic histoarchitecture (Plate 2-11, 13-22, and 24-30).

Table 11 Effect of aqueous extract of the aerial parts of *Leonurus cardiaca* on the plasma lipid profile of normal wistar albino rats

Treatment	Total Cholesterol (mg/dl)	HDL-CHOL (mg/dl)	LDL-CHOL (mg/dl)	VLDL-CHOL (mg/dl)	Triglyceride (mg/dl)
Normal control	56.65±0.01	36.75±0.01	76.89±0.01	10.65±0.01	52.14±0.01
166mg/kg for 7 days	56.92±0.01[a]	36.87±0.01	76.43±0.01[a]	10.05±0.02[a]	52.44±0.02[a]
166mg/kg for 14 days	57.44±0.01[a]	36.94±0.01	76.04±0.01[a]	9.76±0.02[a]	52.85±0.01[a]
166mg/kg for 21 days	57.94±0.02[a]	37.25±0.01[a]	75.94±0.01[a]	9.74±0.02[a]	53.25±0.01[a]
250mg/kg for 7 days	61.01±0.01[a]	43.55±0.01[a]	73.85±0.02[a]	9.15±0.02[a]	53.85±0.01[a]
250mg/kg for 14 days	61.73±0.01[a]	43.94±0.01[a]	73.24±0.01[a]	8.25±0.01[a]	54.24±0.01[a]
250mg/kg for 21 days	73.25±0.01[a]	48.13±0.02[a]	67.83±0.01[a]	8.05±0.01[a]	58.23±0.01[a]
500mg/kg for 7 days	83.24±0.02[a]	53.84±0.01[a]	61.44±0.02[a]	7.23±0.01[a]	64.74±0.01[a]
500mg/kg for 14 days	87.05±0.02[a]	55.24±0.01[a]	58.76±0.01[a]	7.08±0.04[a]	68.04±0.01[a]
500mg/kg for 21 days	93.09±0.02[a]	60.02±0.01[a]	53.74±0.01[a]	6.84±0.01[a]	57.82±0.01[a]

Values are reported as mean ± standard error of mean (M±SEM) (n =5). Values with similar superscript letters indicate significant differences ($p\leq 0.05$) when compared to the control group.

Improvement on the heart tissues was observed after treatment with aqueous extract of the aerial parts of *Leonurus cardiaca* for 7, 14, and 21 days when compared to the control heart, kidney and liver tissues (Plates 1-10). Appearance of preventricular inflammation and normal myocytes

(blue arrows), mild perivascular inflammation and normal myocytes (black arrows), and improved perivascular inflammation and normal myocytes (Plates 1-10). The improvement and mild inflammation after treated with Leonurus cardiaca extract is indicative of non-toxic effect of the extract on heart tissues (Plates 1-10).

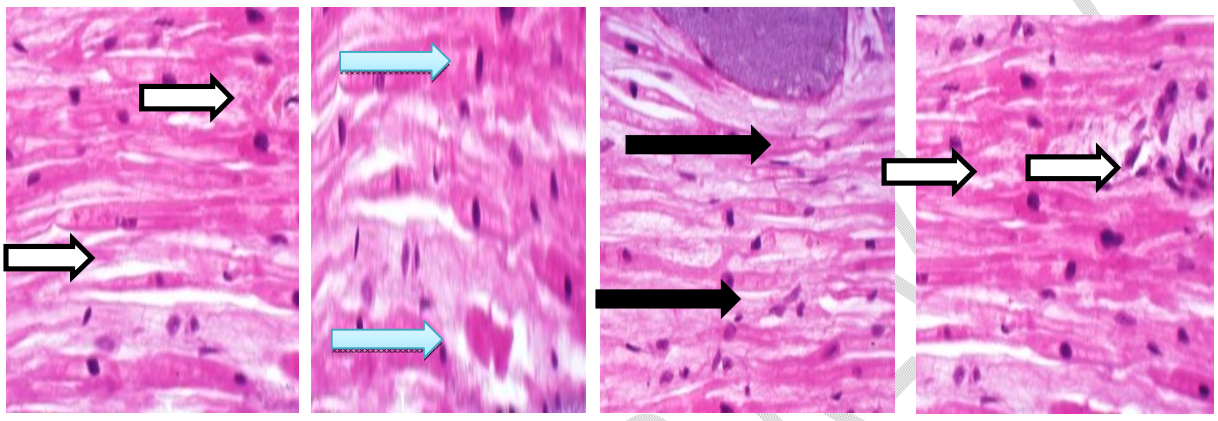

Plate 1 Plate 2 Plate 3 Plate 4

Plate 1: Photomicrograph of positive control showing normal myocytes (white arrows)

Plate 2: Photomicrograph of normal wistar albino rats exposed to 166 mg/kg b.wt of aqueous extract of the aerial parts of *Leonurus cardiaca* for 7 days for acute toxicity testing, showing, preventricular inflammation and normal myocytes (blue arrows).

Plate 3: Photomicrograph of normal wistar albino rats exposed to 166 mg/kg b.wt of aqueous extract of the aerial parts of *Leonurus cardiaca* for 14 days for acute toxicity testing, showing, mild perivascular inflammation and normal myocytes (black arrows).

Plate 4: Photomicrograph of normal wistar albino rats exposed to 166 mg/kg b.wt of aqueous extract of the aerial parts of *Leonurus cardiaca* for 14 days for acute toxicity testing, showing, mild perivascular inflammation and normal myocytes (white arrows).

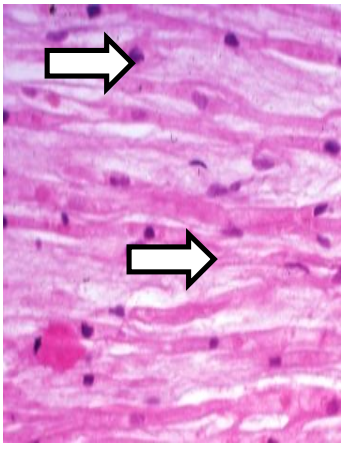

Plate 5

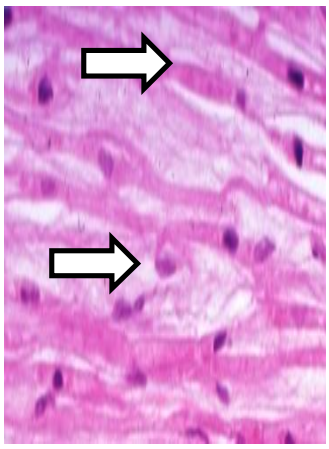

Plate 6

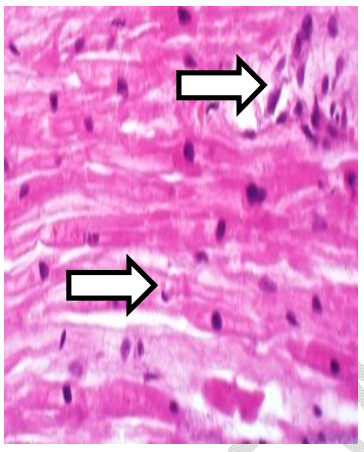

Plate 7

Plate 5: Photomicrograph of normal wistar albino rats exposed to 250 mg/kg b.wt of aqueous extract of the aerial parts of *Leonurus cardiaca* for 7 days for acute toxicity testing, showing, mild perivascular inflammation and intracellular fatty accumulation.

Plate 6: Photomicrograph of normal wistar albino rats exposed to 250 mg/kg b.wt of aqueous extract of the aerial parts of *Leonurus cardiaca* for 14 days for acute toxicity testing, showing, mild perivascular inflammation and intracellular fatty accumulation.

Plate 7: Photomicrograph of normal wistar albino rats exposed to 250 mg/kg b.wt of aqueous extract of the aerial parts of *Leonurus cardiaca* for 21 days for acute toxicity testing, showing, mild perivascular inflammation and intracellular fatty accumulation.

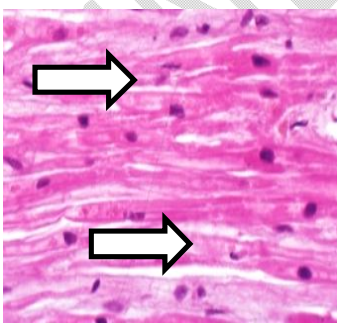

Plate 8

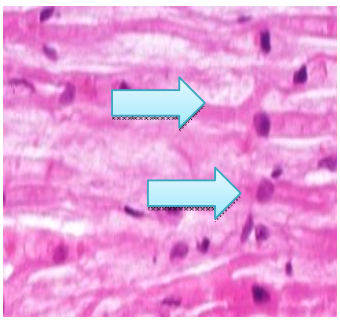

Plate 9

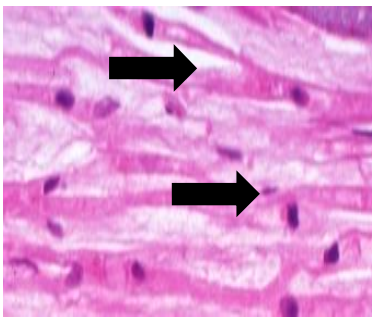

Plate 10

Plate 8: Photomicrograph of normal wistar albino rats exposed to 500 mg/kg b.wt of aqueous extract of the aerial parts of *Leonurus cardiaca* for 7 days for acute toxicity testing, showing, mild perivascular inflammation and normal myocytes.

Plate 9: Photomicrograph of normal wistar albino rats exposed to 500 mg/kg b.wt of aqueous extract of the aerial parts of *Leonurus cardiaca* for 14 days for sub-acute toxicity testing, showing, norma perivascular inflammation and normal myocytes.

Plate 10: Photomicrograph of normal wistar albino rats exposed to 500 mg/kg b.wt of aqueous extract of the aerial parts of *Leonurus cardiaca* for 21 days for acute toxicity testing, showing, improved perivascular inflammation and normal myocytes.

However, treatment with aqueous extract of the aerial parts of Leonurus cardiaca resulted in improvement on the kidney tissues when compared to the control (Plates 11-20). The presentation of normal kidney tissues after treatment with the extract for 7, 14, and 21 days is suggestive of the non-nephrotoxic effect of aqueous extract of the aerial parts of *Leonurus cardiaca* (Plates 11-20).

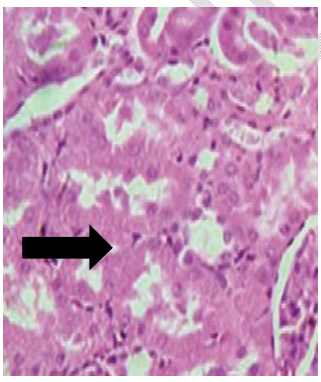

Plate 11

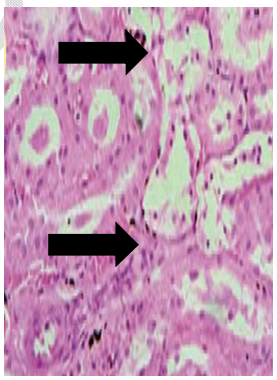

Plate 12

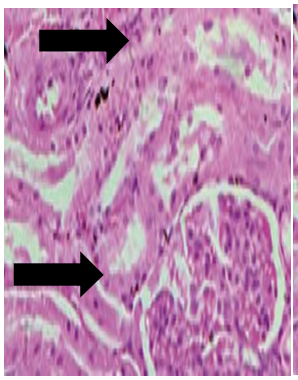

Plate 13

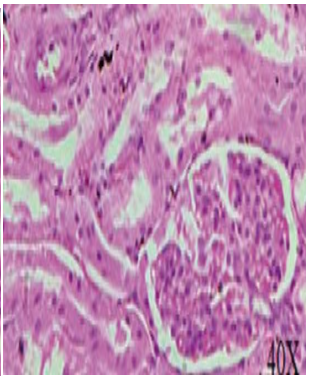

Plate 14

Plate 11: Control Plate 12: 166 mg 7 days Plate 13: 166 mg 14 Plate 14: 166 mg 21 days, showing normal kidney architecture

Plate 12: Photomicrograph of normal in wistar albino rats exposed to 166 mg/kg b.wt of aqueous extract of the aerial part of *Leonurus cardiaca* for 7 days, for toxicity study of the extracts, showing normal kidney tissues.

Plate 13: Photomicrograph of normal in wistar albino rats exposed to 166 mg/kg b.wt of aqueous extract of the aerial part of *Leonurus cardiaca* for 14 days, for acute toxicity study of the extract, showing normal kidney tissues.

Plate 14: Photomicrograph of the kidney of normal in wistar albino rats exposed to 166 mg/kg b.wt of aqueous extract of the aerial part of *Leonurus cardiaca* for 21 days for chronic toxicity study of the extract, showing normal kidney tissues.

Plate 14: Photomicrograph of the kidney of normal in wistar albino rats exposed to 166 mg/kg b.wt of aqueous extract of the aerial part of *Leonurus cardiaca* for 21 days for chronic toxicity study of the extract, showing normal kidney tissues.

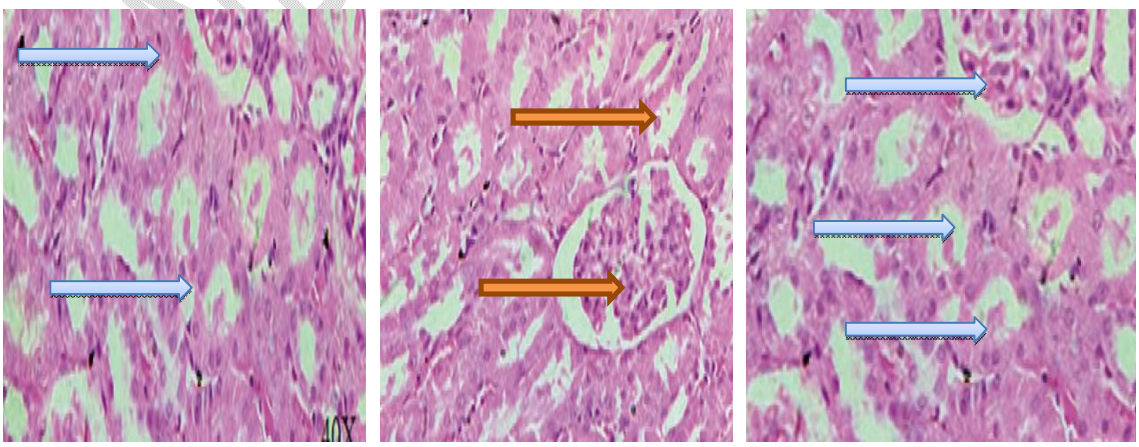

Plate 15 Plate 16 Plate 17

Plate 15: Photomicrograph of the kidney of normal in wistar albino rats exposed to 250 mg/kg b.wt of aqueous extract of the aerial part of *Leonurus cardiaca* for 7 days, for acute toxicity study of the extract, showing normal kidney tissues.

Plate 16: Photomicrograph of the kidney of normal in wistar albino rats exposed to 250 mg/kg b.wt of aqueous extract of the aerial part of *Leonurus cardiaca* for 14 days, for sub-acute toxicity study of the extract, showing normal kidney tissues.

Plat 17: Photomicrograph of the kidney of normal in wistar albino rats exposed to 250 mg/kg b.wt of aqueous extract of the aerial part of *Leonurus cardiaca* for 21 days, for chronic toxicity of the extract, showing normal kidney tissues.

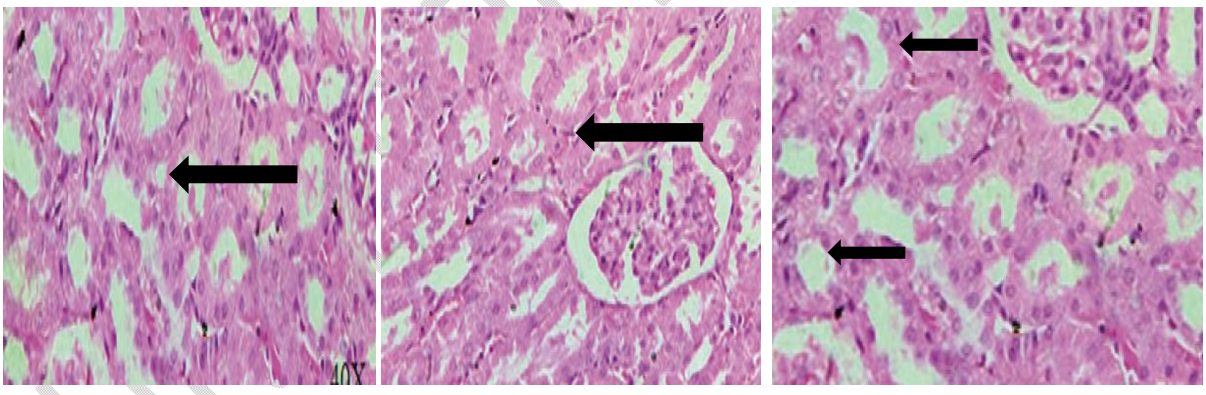

Plate 18 Plate 19 Plate 20

Plate 18: Photomicrograph of the kidney of normal in wistar albino rats exposed to 500 mg/kg b.wt of aqueous extract of the aerial part of *Leonurus cardiaca* for 7 days, for acute toxicity testing of the extract, showing normal kidney tissues.

Plate 19: Photomicrograph of normal in wistar albino rats exposed to 500 mg/kg b.wt of aqueous extract of the aerial part of *Leonurus cardiaca* for 14 days for sub-acute toxicity study of the extract, showing normal kidney tissues.

Plate 20: Photomicrograph of the kidney of normal in wistar albino rats exposed to 500 mg/kg b.wt of aqueous extract of the aerial part of *Leonurus cardiaca* for 21 days chronic toxicity study of the extract, showing normal kidney tissues.

Plate 20: Photomicrograph of normal in wistar albino rats exposed to 500 mg/kg b.wt of aqueous extract of the aerial part of *Leonurus cardiaca* for 14 days for sub-acute toxicity study of the extract, showing normal kidney tissues and similar improvement was observed on the liver tissues as shown in plates 21-30.

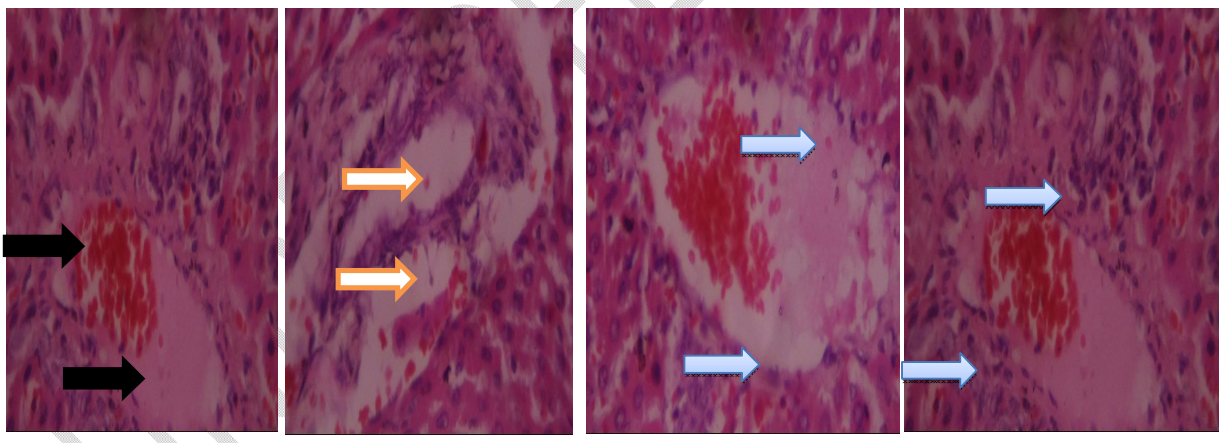

Plate 21 Plate 22 Plate 23 Plate 24

Plates 21-24: Photomicrograph of the liver tissue of normal rats, treated with 166 mg/kg of aqueous extract of the aerial parts of *Leonurus cardiaca* for 7, 14, and 21 days respectively (H&E) staining) x 400.

Plate 21: Photomicrograph of liver tissue of normal control showing normal liver histology.

Plate 22: Photomicrograph of liver tissue of normal rats, treated with 166 mg/kg of aqueous extract of the aerial parts of *Leonurus cardiaca* for 7 days, showing inflammation and normal liver histology.

Plate 23: Photomicrograph of liver tissue of normal rats, treated with 166 mg/kg of aqueous extract of the aerial parts of *Leonurus cardiaca* for 14 days, showing improved liver histology.

Plate 24: Photomicrograph of liver tissue of normal rats, treated with 166 mg/kg of aqueous extract of the aerial parts of *Leonurus cardiaca* for 21 days, normal liver histology.

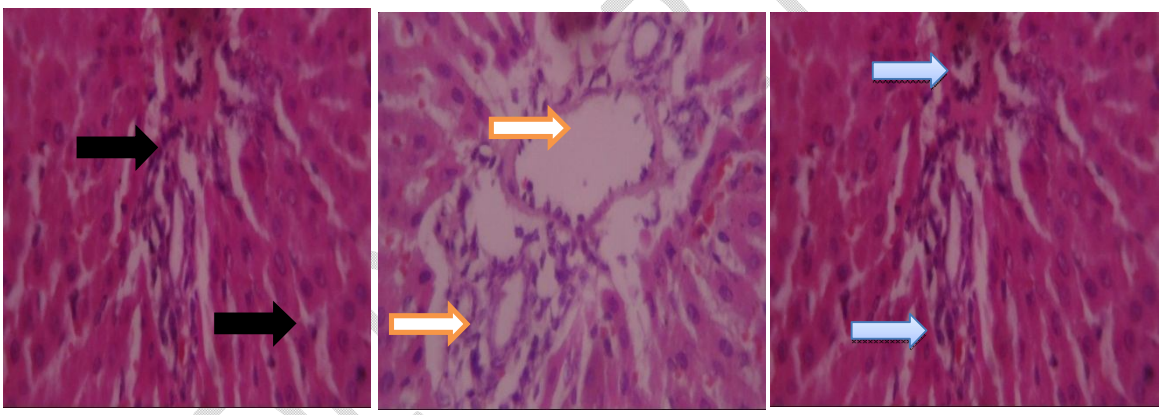

Plate 25 Plate 26 Plate 27

Plates 25-27: Photomicrograph of the liver tissue of normal rats, treated with 250 mg/kg of aqueous extract of the aerial parts of *Leonurus cardiaca* for 7, 14, and 21 days respectively (H&E) staining) x 400.

Plate 25: Photomicrograph of liver tissue of normal rats, treated with 250 mg/kg of aqueous extract of the aerial parts of *Leonurus cardiaca* for 7 days, showing improved liver histology.

Plate 26: Photomicrograph of liver of normal rats treated with 250 mg/kg of aqueous extract of the aerial parts of *Leonurus cardiaca* for 14 days, showing mild inflammation and improved liver architecture.

Plate 27: Photomicrograph of liver of normal rats treated with 250 mg/kg of aqueous extract of the aerial parts of *Leonurus cardiaca* for 21 days, showing improved liver histology.

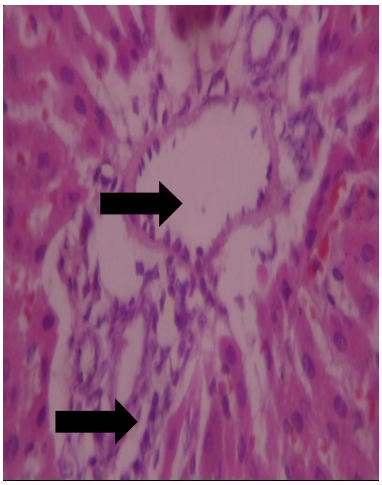

Plate 28

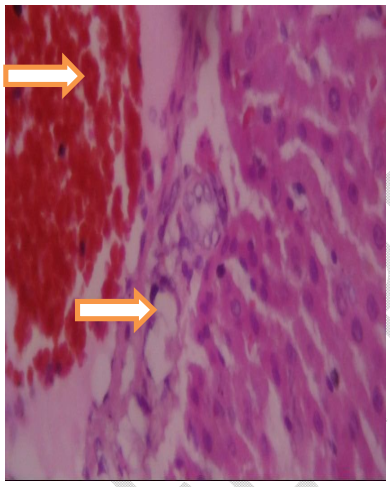

Plate 29

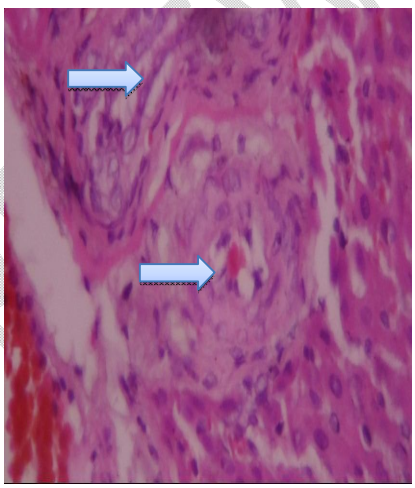

Plate 30

Plates 28-30: Photomicrograph of the liver tissue of normal rats, treated with 500 mg/kg of aqueous extract of the aerial parts of *Leonurus cardiaca* for 7, 14, and 21 days respectively (H&E) staining) x 400.

Plate 28: Photomicrograph of liver of normal rats, treated with 500 mg/kg of aqueous extract of the aerial parts of *Leonurus cardiaca* for 7 days showing normal liver histology.

Plate 29: Photomicrograph of liver of normal rats treated with 500 mg/kg of aqueous extract of the aerial parts of *Leonurus cardiaca* for 14 days showing normal liver histology.

Plate 30: Photomicrograph of liver of normal rats treated with 500 mg/kg of aqueous extract of the aerial parts of *Leonurus cardiaca* for 21 days, showing almost normal liver histology.

Conclusion

In the light of finding of acute, sub-acute and chronic toxicity studies of aqueous extract of *Leonurus cardiac*, as per 425 indicated improvement on all assayed biomarkers, haematological profile and heart, kidney and liver tissues. The significantly improved effect observed from this study is expressive that aqueous extract of the aerial parts of *Leonurus cardiaca* elicited non-toxic effects and the plant extract is safe at 166, 250, and 500 mg/kg.

ETHICAL APPROVAL

All authors hereby declared that the principles of laboratory animal care were followed as well as scientific national laws where applicable. All experiments and procedures were thoroughly examined and approved by the ethical committee on human and animal research University of Port Harcourt.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

NOTE

This study highlighted the effectiveness of "traditional medicine" which is an ancient tradition practiced in some parts of India. This ancient concept should be carefully investigated in the light of modern clinical science and can be adopted partially if considered appropriate

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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